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BRAIN LEVELS OF 5-HYDROXYTRYPTAMINE (SEROTONIN) AFTER ACUTE AND CHRONIC ADMINISTRATION OF LYSERGIC ACID DIETHYLAMIDE (LSD-25)

Robert L. Fisher

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BRAIN LEVELS OF 5-HYDROXYTRYPTAMINE (SEROTONIN) AFTER ACUTE AND CHRONIC ADMINISTRATION OF LYSERGIC ACID DIETHYLAMIDE (LSD-25)

by

Robert L. Fisher, II

Thesis presented to the Faculty of Yale University, School of Medicine in candidacy for the degree of Doctor of Medicine.

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PURPOSE

Since the discovery that 5-hydroxytryptamine ("Serotonin" or 5-HT) occurs naturally in significant amounts in brain tissue, evidence has accumulated from many investigations to suggest that it may play a role in neural functioning. Of particular interest has been the observation that lysergic acid diethylamide (LSD-25), a substance known to produce hallucinatory effects in man, strongly antagonizes the pharmacological actions of 5-hydroxytryptamine (5-HT) on a number of peripheral tissues. Some workers have postulated that the psychotomimetic actions of LSD-25 may be mediated through a central antagonism of 5-HT. Intensive studies up to the present time, however, have failed to define clearly the neuropharmacological interactions between LSD-25 and 5-hydroxytryptamine (5-HT).

Profound alterations in brain levels of 5-HT have been shown to follow the administration of certain drugs (reserpine, iproniazid, and 5-hydroxytryptophan), which affect mental processes. These agents also have certain structural and pharmacologic relationships in common with 5-HT. These findings suggest the possibility that similar changes in brain 5-HT may occur with LSD-25--particularly if a central interaction between LSD-25 and 5-HT takes place. In earlier reports in the literature, 5-HT levels in rat brains were found to be unaltered four hours following a single injection of LSD-25. These investigations, however, failed to account for changes in 5-HT brain levels which might be detectable at shorter or longer periods following LSD-25 administration. Moreover, the possibility of changes in 5-HT distribution in specific brain areas was not

explored. The measurement of 5-HT brain levels following chronic administration of LSD-25 has not been reported although unpublished data in this laboratory showed variably increased levels of 5-HT in the brains of rats rendered tolerant to the mental effects of LSD-25 by repeated daily injections.

The present work has been undertaken to study the general problem of the central interaction between LSD-25 and 5-HT by furthering the measurements of previous investigators of brain levels of 5-HT following acute and chronic administration of LSD-25. Using this technique, the contention that the central actions of LSD-25 are mediated through its interaction with 5-HT, might be supported by the detection of alteration of 5-HT levels following single doses of LSD-25. The finding of changes in 5-HT brain level only after chronic administration of LSD-25 would suggest that the tolerance, which occurs to certain actions of LSD-25 on repeated administration, might be related to a central interaction between the two compounds.

The investigation will be limited specifically to the following:

1. The determination of serotonin (5-HT) content in whole rat brains at varying time intervals following a single injection of LSD-25.
2. The determination of 5-HT levels in whole brains of rats rendered partially or completely tolerant to LSD-25--ten minutes and twenty-four hours following the last injection of LSD-25.
3. The determination of 5-HT levels in certain discreet areas of the dog's brain following acute and chronic administration of LSD-25.

HISTORICAL BACKGROUND

I. 5-Hydroxytryptamine and the Nervous System:

5-Hydroxytryptamine (5-HT) was first isolated from natural sources and identified only a few years ago through the independent investigations of Rappert et al. and Erspamer et al.[1,2] Subsequently it has been found to have widespread occurrence in nature.[3] In vertebrates, 5-HT is present in significant quantities in the gastrointestinal mucosa, platelets, spleen and nervous tissue.[3] Many pharmacological properties have been described for 5-HT and are reviewed at length by Erspamer[3] and Page.[4,5] Of these pharmacological actions of 5-HT, stimulation of certain smooth muscle is the most characteristic.

Owing to its widespread distribution and high pharmacological activity, 5-hydroxytryptamine has evoked a great deal of study and speculation as to its biologic significance. It was postulated by early workers that 5-HT acted to (1) influence hemostasis, (2) control vascular tone and (3) participate in the regulation of the function of the kidney.[3] The hypothesis was then proposed in 1953 by Gaddum[6] and in 1954 by Woolley and Shaw[7,8] that 5-HT participates actively in the physiology of the nervous system. Though its precise role is at present uncertain, much experimental evidence in recent years supports the latter postulate. 5-HT has been shown to possess many of the properties which one would attribute to a substance partaking in neural functioning. This evidence, which will be discussed in detail, consists briefly of the following:

(A) 5-HT occurs in significant amounts in the nervous system; (B) Enzymes for the synthesis and destruction of 5-HT are also present in the nervous system; (C) 5-HT has direct actions on neural functioning: (D) 5-HT may be released during nervous activity; (E) Some compounds having actions on the nervous system are biochemically or pharmacologically related to 5-HT and may possibly act through 5-HT.

A. Occurrence of 5-HT in the Nervous System: 5-Hydroxytryptamine was first demonstrated in brain extracts by Twarog and Page[9] in 1953 and its presence was confirmed by Zetler and Schlosser in 1954.[10] These workers utilized an acetone extraction procedure and bioassay with the heart of Venus mercenaria. Subsequently, Amin et al.[11] and Paasonen and Vogt[12] made the interesting observation that 5-HT is not equally distributed in the brain of the dog but discreetly localized in certain brain structures. The highest concentrations of 5-HT were found in brain nuclei associated with autonomic function--the hypothalamus, midbrain and medulla, and in the area postrema, while no detectable amounts were present in neo-cortical areas or medullary nerve fibers. The general pattern of distribution of 5-HT was noted to parallel that found by Vogt for nor-epinephrine.[13] Paasonen, McLean and Giarman showed that certain limbic cortical structures--the medial and lateral pyriform cortex, the entorhinal cortex and hippocampus also contain appreciable concentrations of 5-HT.[14] More recently, Udenfriend et al. were able to determine 5-HT brain levels by direct measurement, using a flourometric assay and butanol extraction.[15-19] Their results revealed a pattern of

distribution of 5-HT resembling those of previous investigators. The ubiquity of 5-HT in the nervous system was demonstrated by its detection in certain peripheral autonomic ganglia by Florey and Florey,[20] Gertner et al.[21] and Welsh.[22]

B. Occurrence of Enzymes for the Synthesis and Destruction of 5-HT in the Nervous System: The synthesis of 5-HT has been shown to procede from tryptophan and to depend upon two enzymes--tryptophan oxidase and 5-hydroxytryptophan decarboxylase.[23,24] Tryptophan is converted to 5-hydroxytryptophan in the presence of tryptophan oxidase.[23] Though this enzyme has been studied only in microorganisms, its apparent absence is animal tissues has been explained by Udenfriend et al. to result from the fact that the above reaction constitutes the rate limiting step in the synthesis.[25] The second enzyme, 5-hydroxytryptophan decarboxylase, specifically catalyzes the decarboxylation of 5-hydroxytryptophan to 5-hydroxytryptamine.[24-26] 5-Hydroxytryptophan decarboxylase has been detected in homogenates of a number of mammalian tissues including nervous tissue by Caddum and Gidrman[27] and by Udenfriend et al.[17,18] The data of the above workers indicate that 5-hydroxytryptophan decarboxylase has a distribution in the dog's nervous system which parallels, in general, that of 5-HT with the exception of the area postrema which contains relatively small amounts of the enzyme, and the sympathetic ganglia which have relatively high decarboxylase activity. The experiments of Bogdanski, Weissbach and Udenfriend further emphasize the important activity of 5-hydroxytryptophan decarboxylase in the brain.[28-31]

They noted that 5-HT produced in the periphery failed to reach the brain because of its destruction in the blood by monoamine oxidase and its failure to penetrate the blood-brain barrier. They were able, however, to produced brain levels of 5-HT several times higher than normal following peripheral injections of 5-hydroxytryptophan. This precursor of 5-HT readily gained access to the brain and was decarboxylated.

Of the numerous possible pathways for the destruction of 5-HT, its oxidation to 5-hydroxyindoleacetic acid is probably the most important.[3,22,32] Monoamine oxidase, the enzyme which catalyzes this reaction, has been clearly demonstrated in brain tissue.[16-19] Unlike 5-HT, however, it has been found to be relatively evenly distributed throughout the brain.[19] Of note is the demonstration that the administration of iproniazid ("Marsilid"), a monoamine oxidase inhibitor, causes significant elevations of 5-HT brain levels.[34,35]

C. Action of 5-hydroxytryptamine (5-HT) on Neural Functioning:

Many different techniques of direct and indirect administration have been employed to study the central actions of 5-HT because of the difficulty in obtaining pharmacological levels in the brain following parenteral injection. On the other hand, certain methods of study have lended themselves to the use of peripheral 5-HT administration. Shore et al. obtained indirect evidence of the central actions of 5-HT using intraperitoneal injections in mice.[36,37] They observed that 5-HT had a striking central potentiating action on certain hypnotics. In electrophysiological experiments, Marrazzi

et al. observed marked cerebral synaptic inhibition in the transcallosal pathway of the visual system of the cat following intracarotid injections of 5-HT.[38-40] Similarly, Everts et al. noted that bufotenine, a 5-HT analog, produced inhibition of the postsynaptic spike in the lateral geniculate nucleus.[41] 5-HT exerted variable effects on the spinal reflexes of the cat following intravenous administration according to the work of von Weidmann and Cerletti.[42,43]

Using the technique of peripheral injection of 5-hydroxytryptophan, as previously described, Bogdanski et al. were able to produce high brain concentrations of 5-HT and observe their effects.[28-31] Small doses of 5-hydroxytryptophan produced sedation while high doses were accompanied by excitement, muscle tremors, loss of placing reactions, loss of response to visual stimulation, and various autonomic effects resembling those produced by LSD-25. These effects were seen to correspond to 5-HT levels in the brain and were presumed, therefore, to be due to the 5-HT formed from 5-hydroxytryptophan rather than to 5-hydroxytryptophan itself.

Several workers have studied the effects of intraventricular injections of 5-HT. By the use of a permanently implanted cannula in a lateral ventricle of the cat, as originally introduced by Feldberg and Sherwood, the observation of conscious behavior following the introduction of 5-HT has been possible.[44] In these studies, 75-500 micrograms of 5-HT have been followed in most instances by signs of depression in animals.[45-47] Vogt et al. observed that intraventricular injections of 5-HT produced low voltage slow activity in the spontaneous

E.E.G. of most of the brain areas studied, although long bursts of high amplitude were noted in the reticular formation.[46]

In anatomical studies, human and rat oligodendroglia were observed in tissue culture to contract in response to 5-HT.[48] On this basis, Woolley and Shaw postulated that 5-HT might be responsible for rhythmic contractions of these cells, and thus play a role in promoting intracerebral circulation.[48]

D. Release of 5-HT during Nervous Activity: Taylor, Corcoran and Page in 1951 provided some evidence that 5-HT might be released following nervous stimulation.[49] Using crossed circulation experiments, they demonstrated the release of a vasopressor substance following central vagal stimulation in spinal dogs. The vasopressor effect was unaffected by various blocking agents but was inhibited by 1-hydrazinopthalazine, a compound which had previously been shown to inhibit the pressor action of 5-HT.

Welsh showed that 5-HT excited the molluscan heart in the same manner as stimulation of the visceral ganglion following blockade of the cholinergic inhibitor nerves.[22,50] Agents that blocked 5-HT also abolished nervous excitation of the heart, suggesting that 5-HT or closely related substance might be released from the nerve endings.

In preliminary experiments with perfused spinal cord of the frog, Angelucci discovered a release of active substances during the course of reflex activity.[51] Though not confirmed, one of the substances in the perfusion fluid resembled 5-HT.

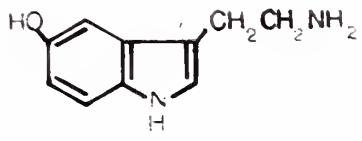
E. Effects of Other Drugs Related to Serotonin (5-HT): Brodie and his co-workers in 1955 made one of the most interesting discoveries in the recent work with 5-HT. They observed that the tranquilizing agent, reserpine, was capable of releasing 5-HT from its binding sites in the brain and other body stores in experimental animals.[52-53] Following an injection of 5 mg./kg. of reserpine, the brain levels of 5-HT declined rapidly such that the release was almost complete in eight hours. Low brain levels of reserpine remained for twenty-four hours and then gradually increased to normal values over the next seven days. During the period in which the brain content of 5-HT was low, 5-hydroxyindoleacetic acid, a metabolic product of 5-HT, was excreted in large amounts in the urine. Hess, Shore and Brodie further demonstrated that the pharmacological effects of reserpine persisted long after it was no longer detectable in the brain.[54] These considerations and the finding of similar potentiating actions of 5-HT and reserpine on hexobarbital anesthesia prompted Brodie et al. to postulate that reserpine exerted its central actions through the release of 5-HT.[53-58] In this regard, the persistence of relatively large amounts of 5-HT in the unbound form rather than the depletion of the total brain content of 5-HT, was considered responsible for the effects of reserpine. The above hypothesis was further strengthened by the finding that serotonin-releasing activity was limited to rauwolfia alkaloids (reserpine, deserpidine, and rescinnamine) which have tranquilizing actions.[58] The situation has been complicated, however, by the recent

discovery of nor-epinephrine release from the brain following reserpine administration.[59] High concentrations of unbound serotonin (5-HT), as produced by iproniazid or 5-hydroxytryptophan injections, have been shown to result in excited behavior rather than sedation.[31,34] This inconsistency with Brodie's hypothesis may be explained, on the other hand, by the assumption that 5-HT is self-inhibitory in high concentrations.[17]

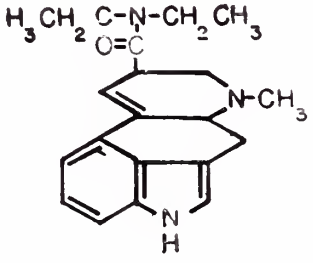
It has been suggested that other drugs may exert their actions on the nervous system through their antagonism with 5-HT.[6-8] Woolley and Shaw noted that a number of drugs, structurally related to 5-HT and pharmacologically antagonistic to its action on smooth muscle, produced mental symptoms.[7-8] (See Fig. 1) They classified these anti-metabolites of 5-HT into three groups: (1) The harmala alkaloids, certain of which produce hallucinations and profound behavioral changes; (2) The ergot alkaloids, including the potent hallucinogenic agent, lysergic acid diethylamide (LSD-25); and (3) Yohimbine, an aphrodisiac. Woolley and Shaw went on to postulate that 5-HT may be required for normal mental functioning and that mental abberations produced by drugs and naturally occurring psycoses may result from a relative 5-HT deficiency. Against this general theory is the strong evidence that (1) not all compounds antagonistic and structurally related to 5-HT produce mental disturbances and that (2) peripheral administration of 5-HT does not correct such disturbances. Further consideration of the postulate of Woolley and Shaw as it regards lysergic acid diethylamide (LSD-25) will take place in later pages.

FIGURE 1

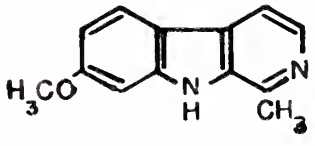
STRUCTURAL FORMULAE OF SEROTONIN AND RELATED COMPOUNDS



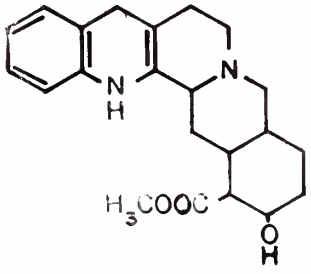
5 HYDROXYTRYPTAMINE



LYSERGIC ACID DIETHYLAMIDE



HARMINE



YOHIMBINE

II. Lysergic Acid Diethylamide, its Properties, and Relationship to 5-HT

Although lysergic acid diethylamide (LSD-25) was first synthesized in 1938, its significance as a psychotogen was not realized until 1943 when Hoffmann accidentally discovered its hallucinogenic properties.[60] Stoll subsequently studied the psychiatric effects of LSD-25 in a number of normal and schizophrenic subjects.[61] The compound, in doses as small as 20--30 micrograms, was observed to produce a state of intoxication of the acute exogenic reaction type, in which optic hallucinations and distortions were prominent.[61] These clinical observations have been confirmed by numerous investigators and recently reviewed by Hoch[62] and Koelle.[63] Aside from its actions on mental processes, LSD-25 exerts many actions on the autonomic nervous system and certain peripheral structures. These pharmacological properties have been considered in the reviews of Rothlin and Cerletti[64-65] and are summarized in Figure 2.

Of the autonomic actions of LSD-25, its pyretogenic property has received considerable interest. Horita and Dille showed that the rectal temperature in rabbits, dogs, and cats rose significantly following intravenous LSD-25 in a dose of 50 micrograms/kilogram.[66] In rabbits the effect was maximal at the end of 1-2 hours but persisted 6-8 hours.[66-67] Experiments using pharmacological techniques and sectioning of the brain at different levels have suggested that the LSD-25 hyperpyrexia is mediated through a central diencephalic site of action.[67-68]

FIGURE 2

THE PHARMACOLOGICAL PROPERTIES OF

LYSERGIC ACID DIETHYLAMIDE (LSD-25)*

SOMATO-MOTOR FUNCTIONS

Pyramidal and Extrapyramidal Effects
Ataxia and Spastic Paralysis

PSYCHIC FUNCTIONS

Excitation
Mood Changes
Disturbances of Perception
Depersonalization
Schizophrenic State

AUTONOMIC NERVOUS FUNCTIONS

Mesodiencephalic Effects
- Mydriasis
- Tachycardia
- Hyperpyrexia
- Hyperglycemia
- Pilomotor Reaction

Medullary or Bulbar Effects
- Lowering of the Blood Pressure
- Bradycardia
- Respiratory Depression

DIRECT PERIPHERAL ACTIONS

Inhibition of 5-HT on Different Organs in Vitro and in Vivo
- Uterus
- Blood Vessels Artificially Perfused
- Bronchial Musculature in High Doses
- Blood Vessels of the Spinal Cat

Adrenolytic Action on Isolated Uterus and Seminal Vesicle

* Adapted from Rothlin, E.: Pharmacology of lysergic acid diethylamide (LSD) and some of its related compounds. J. of Pharmacy and Pharmacology, 9: 569, 1957.

The phenomenon of tolerance has been repeatedly observed in regard to several actions of LSD-25 following repeated administration of the compound. Stoll first described the development of tolerance to the psychic actions of LSD-25 in schizophrenic patients.[61] More recently, tolerance to the hallucinatory actions in normal subjects and to the behavioral changes in schizophrenic patients has been observed to develop within four or five days with daily administration of LSD-25.[69-73] Similarly, tolerance has been observed with LSD-25 induced behavioral aberations in animals.[74-76] Gogerty and Dille described tolerance to the pyretogenic effects of LSD-25 in rabbits.[77] Of note has been the observation that rats do not develop tolerance to LSD-25-induced bradycardia.[75]

Owing to the stimulus of the suggestion of Gaddum,[6] and Woolley and Shaw[7,8] that LSD-25 might exert its central actions through an antagonism of 5-HT, considerable study of the interactions of 5-HT and LSD-25 has taken place. Regarding peripheral sites of action, Gaddum has shown that LSD-25 in very small doses exerts a specific antagonism to the action of 5-HT on the rat uterus.[6,78] LSD-25 has also been noted to antagonize the vasodilating action of 5-HT on the perfused rabbit ear.[79-80] Gaddum and Hameed observed little antagonism between LSD-25 and 5-HT in experiments on guinea pig ileum but pointed out that a different type of tryptamine receptor in the autonomic ganglia of the intestine would account for the apparent lack of interaction.[80] On the other hand, some investigators, notably Woolley and Shaw[81] and Welsh and McCoy,[82] have observed similarities

in the actions of LSD-25 and 5-HT on certain peripheral structures.

At the present time, the relationships between LSD-25 and 5-HT regarding their actions on the central nervous system are not clear. The hypothesis of Woolley and Shaw received some support in the studies of Shore et al. demonstrating the potent antagonistic action of LSD-25 on the potentiation of hexobarbital hypnosis in mice by 5-HT.[36-37] However, considerable evidence has accumulated to indicate that the peripheral antagonism of certain actions of 5-HT by LSD-25, does not by itself, explain the central effects of LSD-25. Rothlin and Cerletti have pointed out that 2-Brom-LSD, a strong peripheral antagonist of 5-HT lacks the central actions of LSD-25.[83] In another study, these investigators showed, using several derivatives of lysergic acid, that the relative potency of 5-HT antagonism was unrelated to psychic effect.[84] Gaddum and Vogt observed sedation and excitement following the intraventricular injection of 5-HT and LSD-25, respectively.[47] Administered together, LSD-25 and 5-HT produced the effects of LSD-25 alone. On the basis of these and electroencephalographic observations, Vogt et al. concluded that the effects of LSD-25 and 5-HT depended upon the action of each drug on separate receptor sites, and not upon a competition for the same receptors.[46] Other neurophysiological investigations have produced conflicting results.[38-40,45,85] Of note has been the finding of apparent similar actions of both compounds on the synaptic potentials of the transcallosal pathway of the cat's visual system,[38-40] and on diencephalic temperature regulation.[86]

METHODS

Two basic groups of experiments were included in this work. In the first group, the rat was used as the experimental animal, and the whole brain was analyzed for serotonin content. In this way, the effect of LSD-25 on total 5-HT content of the brain could be determined with variation in several different parameters. The second group utilized dogs treated with LSD-25 in order to detect more subtle changes in 5-HT content within specific brain areas.

I. Experiments on 5-HT in Whole Rat Brain:

Male albino rats weighing 100-250 grams were used. LSD-25 was administered intraperitoneally in a dose of 100 micrograms/kilogram. In the first experiment, three rats served as controls, while the LSD-25 treated rats were sacrificed at various time intervals (0.5, 1, 2, 4, and 8 hours) following a single LSD-25 injection. The second and third experiments involved the daily administration of LSD-25 over the period of five days. On each day, three LSD-25 treated rats were sacrificed at 10 minute and 24 hour intervals following the LSD-25 injection, respectively. Five control rats were included in each experiment. The time intervals of 10 minutes and 24 hours were chosen as perhaps being the most likely times to detect 5-HT changes, since the maximum behavioral effects of LSD-25 in the rat usually occur in 10-15 minutes,[76/] and since tolerance, which occurs with daily injections of LSD-25, presumably is preceded by biochemical changes in the brain which are present 24 hours after the previous

injection. All rats were sacrificed by decapitation. The brains (excluding the olfactory bulbs and pituitary glands) were rapidly dissected out, weighed and homogenized in 4 ml. of isotonic saline. The 5-HT extraction and assay are described below.

II. Experiments on 5-HT in Specific Areas of the Dog's Brain:

For these experiments mongrel dogs of both sexes were used. Four dogs served as controls and did not receive LSD-25. The remainder were treated with intraperitoneal injections of LSD-25 in doses of 25 micrograms/kilogram or 50 micrograms/kilogram as noted in the results. Two animals received only one LSD-25 injection, three received two daily injections, and two were given daily injections for four days. Rectal temperature observations were made in the latter two animals. The experimental animals were sacrificed 30-60 minutes after the final injection, at which time the maximum behavioral effects of LSD-25 were usually manifested.

All dogs were sacrificed by exsanguination under light chloroform-ethyl chloride anesthesia. The brains were removed and the various brain structures were dissected as described below. The total time required for removal and dissection of the brain was 45 minutes to 1 hour. The brain samples were then weighed, homogenized in isotonic saline and extracted as described below.

III. Dissection of the Dog's Brain:

The brain areas selected to be assayed for 5-HT content were those regions shown by other investigators to contain relatively large amounts of 5-HT or those regions whose function would seem to

be altered by LSD-25 administration. The dissection of most of the brain parts followed clearly-defined anatomical boundaries as outlined in Figure 3. Further description is necessary regarding certain areas, however. The "hypothalamus" sample included the mamillary bodies and infundibulum but did not include the optic chiasm or hypophysis. The "medial thalamus" consisted of a 1 mm. thickness of the medial aspect of the thalamus. The "lateral thalamus" constituted the remainder of the thalamus. The "midbrain" included the corpora quadrigemina and cerebral peduncles. The amygdala nucleus was difficult to visualize anatomically in its entirety and consequently the "amygdala" samples were obtained from only portions of the nuclear gray matter underlying the pyriform cortex.

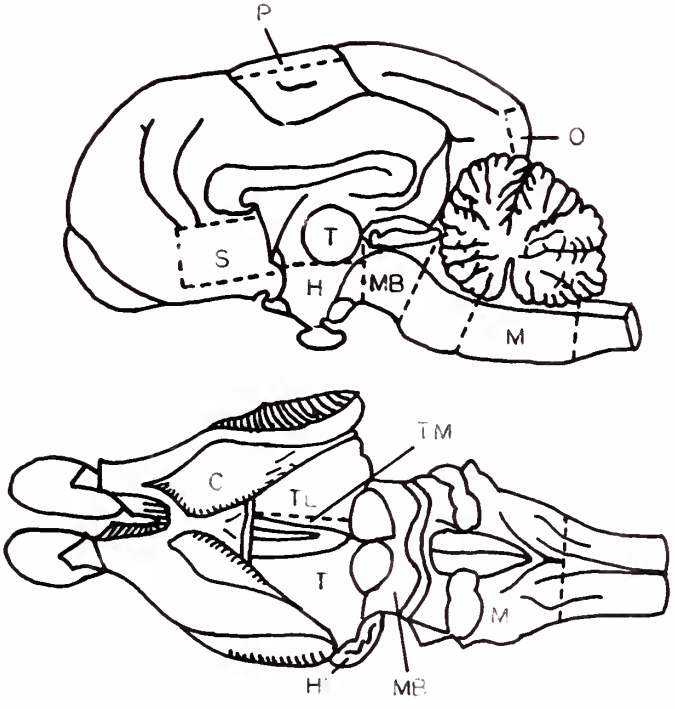
IV. Extraction and Assay of 5-HT:

The homogenates of all brain samples were extracted with 95% acetone according to the method of Amin et al.[11] The 5-HT content of the samples was then estimated by a bioassay procedure using the heart of Venus mercenaria.[9] The heart was bathed at room temperature in artificial sea water containing in grams/liter: NaCl, 23.0; KCl, 0.65; $CaCl_2$, 1.1; $MgCl_2.6\ H_2O$, 5.0; and $NaHCO_3$, 0.2. To prevent the cardiac inhibitory effects of any acetyl choline which might be present in the extracts, benzoquinonium chloride (6 mg./L.) was always added to the bathing medium. Standard solutions of 5-HT and tissue extracts were added alternately to the bathing medium at about 2.5 minute intervals and allowed contact with the heart for about 30 seconds. 5-HT activity of the extracts was estimated from maximal effects of each on the contractile force of the clam's heart.

FIGURE 3

THE DISSECTION OF THE DOG'S BRAIN *

(MIDSAGITAL AND DORSAL VIEWS)



KEY

C--CAUDATE
H--HYPOTHALAMUS
HI--HIPPOCAMPUS
M--MEDULLA
MB--MIDBRAIN
O--OCCIPITAL POLAR CORTEX
P--POSTERIOR SIGMOID CORTEX
S--SEPTAL REGION
T--THALAMUS
TM--MEDIAL THALAMUS
TL--LATERAL THALAMUS

* Adapted from Papez, James W. : Comparative Neurology. Norwood, Thomas Y. Crowell Company, 1929, pp. 20; 128.

RESULTS

I. Effect of a Single LSD-25 Injection upon the Amount of 5-HT in Whole Brains of Rats:

LSD-25 in 100 micrograms/kilogram doses produced only slight observable hyperactivity in the rats tested for 5-HT levels; measures of behavior were not undertaken. The effect of LSD-25 on the content of 5-HT at various time intervals after a single injection is illustrated in Table 1. The values obtained in the experimental animals do not differ by more than twice the standard deviation from the control mean ($p > 0.05$). This indicates that a single injection of LSD-25 does not produce any statistically significant changes in 5-HT content at the time intervals studied.

II. Effect of Chronic Administration of LSD-25 Upon the Amount of 5-HT in the Whole Brains of Rats:

The brain levels of 5-HT obtained by sacrificing rats at 10 minute and 24 hour intervals following daily injections of LSD-25 are represented in Table 2. The results in both experiments suggest a slight depression of 5-HT level after two LSD-25 injections and elevations of 5-HT levels after 3, 4, or 5 daily injections. These values are suggestive of alterations in brain levels of 5-HT but do not show significance by the statistical tests employed.

III. 5-HT Levels in Various Regions of the Untreated Dog's Brain:

The distribution of 5-HT obtained in the brains of control dogs is illustrated in Table 3. The pattern of distribution of 5-HT obtained in these experiments is in general agreement with the results

TABLE 1

SEROTONIN (5-HT) LEVELS IN WHOLE BRAINS OF RATS FOLLOWING A SINGLE INJECTION OF LYSERGIC ACID DIETHYLAMIDE (LSD-25)*

NUMBER OF ANIMALS	TIME OF SACRIFICE (HRS. AFTER TREATMENT)	5-HT (NG./GM. OF BRAIN TISSUE)	MEAN
13	POOLED CONTROLS	355; 452; 326; 326; 390; 409; 460; 445; 226; 191; 355; 482; 399	368**
4	0.5	335; 581; 455; 428	450
3	1	482; 352; 237	357
4	2	337; 290; 359; 329	329
4	4	426; 460; 468; 292	412
2	8	432; 294	363
3	24	345; 368; 491	401

* Dosage of 100 micrograms/kilogram.

** Standard deviation of the mean--85.

TABLE 2

SEROTONIN (5-HT) LEVELS IN WHOLE BRAINS OF RATS AFTER CHRONIC ADMINISTRATION OF LYSERGIC ACID DIETHYLAMIDE (LSD-25) (100 MCG/KG.)

EXPERIMENT A *

NUMBER OF ANIMALS	NUMBER OF DAILY LSD-25 INJECTIONS	5-HT LEVELS (NG./GM. OF BRAIN TISSUE	MEAN
13	POOLED CONTROLS	355; 452; 326; 326; 390; 409; 460; 445; 226; 191; 355; 482; 399	368
3	1	375; 397; 368	380
3	2	239; 242; 244	242
3	3	460; 457; 471	463
3	4	471; 408; 460	446
3	5	302; 302; 393	332

EXPERIMENT B **

NUMBER OF ANIMALS	NUMBER OF DAILY LSD-25 INJECTIONS	5-HT LEVELS (NG./GM. OF BRAIN TISSUE	MEAN
13	POOLED CONTROLS	355; 452; 326; 326; 390; 409; 460; 445; 226; 191; 355; 482; 399	368
3	1	364; 349; 229	314
3	2	245; 231; 231	236
3	3	674; 414; 370	486
3	4	447; 357; 238	347
3	5	600; 479; 473	519

* Rats sacrificed 10 minutes following the last LSD-25 injection.
** Rats sacrificed 24 hours following the last LSD-25 injection.

TABLE 3

SEROTONIN (5-HT) LEVELS IN VARIOUS PARTS OF THE BRAINS OF CONTROL DOGS EXPRESSED AS NG./GM. OF BRAIN TISSUE

BRAIN AREA	5-HT CONTENT	MEAN
MEDULLA	135; 500; 258; 280	294
MIDBRAIN	- ; 2087*; 382; 706	544
POSTERIOR SIGMOID CORTEX	- ; - ; 38; 45; 56	46
OCCIPITAL POLAR CORTEX	- ; <25; <20; 28	<28
SEPTAL REGION	195; 88; 86; 133	126
HYPOTHALAMUS	909; 652; 244; 833	660
HIPPOCAMPUS	167; 92; 100; 300	165
THALAMUS (MEDIAL)	400; 369; 139; 526	358
THALAMUS (LATERAL)	328; 160; 66; 341	224
THALAMUS (WHOLE)	303; 169; 112; 341	231
CAUDATE	244; 214; 145; 283	222
AMYGDALA	444; 308; 313; 469	384

* Due to a technical error; this result was not averaged with the other midbrain values.

of other workers.[11-12, 14, 16-19] The largest amounts of the amine were concentrated in those structures associated with autonomic function. This is particularly well demonstrated in the relatively large quantity of 5-HT contained in the medial thalamus as compared with the lateral thalamus. The highest concentrations of 5-HT were usually obtained in the hypothalamus and midbrain while comparatively small amounts were detected in the neocortical areas (posterior sigmoid cortex and occipital polar cortex.) The remaining brain areas occupied an intermediate position.

IV. Effect of Administration of LSD-25 on 5-HT Levels in the Dog's Brain:

The injection of LSD-25 in doses of 25-50 micrograms/kilogram produced variable behavioral changes in the dogs used in these experiments. No detectable behavioral changes could be noted in some of the animals while others showed a marked response which consisted of: 1. "perking up" the ears; 2. assuming a fixed stance and staring as if out of contact with their surroundings; 3. hyperreacting or hyporeacting to visual and auditory stimuli; and 4. salivating excessively. Dilatation of the pupils was observed in all dogs following the injection of LSD-25. The response to LSD-25 usually was maximal by 30 minutes after the injection and subsided almost completely by the end of four hours. In the two animals treated with 4 daily injections of LSD-25, tolerance was observed to develop to the behavioral and pupillary effects of LSD-25 during the course of the experiment such that little or no response to the LSD-25 was

observable on the fourth day. Rectal temperatures in these two animals showed a mean rise of 0.8^{0}F, 1.05^{0}F and 0.2^{0}F at 30 minutes, 60 minutes and 120 minutes after one LSD-25 injection, respectively. This LSD-25 induced hyperpyrexia was noted to subside during the four days of the experiment. A third dog given 5 daily injections developed tolerance to the behavioral and pupillary changes but not to the temperature effects.

The 5-HT levels in the various parts of the brains of dogs treated with 1, 2 and 4 daily injections of LSD-25 are tabulated in Tables 4, 5, and 6, and are summarized in Table 7. There is no change in the rank order of the various brain areas with respect to 5-HT content in treated and non-treated animals. There is a distinct trend toward elevation of brain 5-HT in all of the LSD-25 treated dogs as compared with the control animals. This trend is seen in all areas but the magnitude of the elevation is most striking in specific areas--the midbrain, hypothalamus and amygdala.

TABLE 4

SEROTONIN (5-HT) LEVELS IN VARIOUS PARTS OF THE DOG'S BRAIN FOLLOWING A SINGLE INJECTION OF LYSERGIC ACID DIETHYLAMIDE (LSD-25) (50 γG./KG.)

BRAIN AREA	5-HT CONTENT*	MEAN*
MEDULLA	417; 463	440
MIDBRAIN	715; 1035	875
POSTERIOR SIGMOID CORTEX	73; 46	60
OCCIPITAL POLAR CORTEX	24; 29	26
SEPTAL REGION	92; 200	146
HYPOTHALAMUS	968; 870	919
HIPPOCAMPUS	212; 334	273
THALAMUS (MEDIAL)	435; 750	592
THALAMUS (LATERAL)	233; 258	246
CAUDATE	164; 400	282
AMYGDALA	606; 577	592

* Expressed as ng./gm. of brain tissue.

TABLE 5

SEROTONIN (5-HT) LEVELS IN VARIOUS PARTS OF THE DOG'S BRAIN FOLLOWING TWO DAILY INJECTIONS OF LYSERGIC ACID DIETHYLAMIDE (EXPRESSED AS NG./GM. OF BRAIN TISSUE)

BRAIN AREA	5-HT CONTENT		MEAN
	DOG A*	DOGS B AND C**	
MEDULLA	328	382; 267	326
MIDBRAIN	800	1176; 826	934
POSTERIOR SIGMOID CORTEX	107	49; 38	65
OCCIPITAL POLAR CORTEX	24	42; 32	33
SEPTAL REGION	250	146; 120	172
HYPOTHALAMUS	833	62; 1364	753
HIPPOCAMPUS	182	188; 125	165
THALAMUS (MEDIAL)	450	643; 300	464
THALAMUS (LATERAL)	454	100; 226	293
THALAMUS (WHOLE)	259	247; 302	269
CAUDATE	522	272; 313	369
AMYGDALA	534	800; 375	570

* Dog A received LSD-25 doses of 50 µg./kg.

** Dogs B and C received LSD-25 doses of 25 µg./kg.

TABLE 6

5-HT LEVELS IN VARIOUS PARTS OF THE DOG'S BRAIN AFTER DAILY ADMINISTRATION OF LSD-25 (50 γG./KG.) FOR FOUR DAYS

BRAIN AREA	5-HT CONTENT*	MEAN
MEDULLA	236; 638	437
MIDBRAIN	674; 1163	918
POSTERIOR SIGMOID CORTEX	73; 77	75
OCCIPITAL POLAR CORTEX	41; 36	38
SEPTAL REGION	109; 155	132
HYPOTHALAMUS	750; 818	784
HIPPOCAMPUS	192; 111	152
THALAMUS (MEDIAL)	588; 417	502
THALAMUS (LATERAL)	179; 164	172
THALAMUS (WHOLE)	- ; 280	280
CAUDATE	221; 286	254
AMYGDALA	465; 450	458

* Expressed as ng./gm. of brain tissue.

TABLE 7

SUMMARY OF MEAN 5-HT LEVELS OF THE DOG'S BRAIN

FOLLOWING CHRONIC DAILY ADMINISTRATION OF LSD-25

(EXPRESSED IN NG./GM. OF BRAIN TISSUE)

BRAIN AREA	5-HT CONTENT			
	CONTROL	1 INJECTION	2 INJECTIONS	4 INJECTIONS
MEDULLA	294	440	326	437
MIDBRAIN	544	875	934	918
POSTERIOR SIGMOID CORTEX	46	60	65	75
OCCIPITAL POLAR CORTEX	<28	26	33	38
SEPTAL REGION	126	146	172	132
HYPOTHALAMUS	660	919	753	784
HIPPOCAMPUS	165	273	165	152
THALAMUS (MEDIAL)	358	592	464	502
THALAMUS (LATERAL)	224	246	293	172
THALAMUS (WHOLE)	231	-	269	280
CAUDATE	222	282	369	254
AMYGDALA	384	592	570	458

DISCUSSION

The results would tend to indicate that no significant alterations of 5-HT levels in the whole rat brain occur at the time intervals studied following a single LSD-25 injection. This is in agreement with the results obtained by other workers examining the brains 4 hours after injection.[14] The experiments involving the chronic administration of LSD-25 to rats suggest slight changes in 5-HT brain content but the results are not statistically conclusive. Similarly slight elevations of 5-HT values were observed generally throughout the areas of the dog's brain following acute and chronic administration of LSD-25. The significance of these slight changes cannot be stated. Certainly, they are not as dramatic as the 2-3 fold increases in brain 5-HT obtained in this laboratory following iproniazid ("Marsalid") administration.[87]

Certain specific details of the experimental method must be considered in evaluating the above results. The possibility exists that changes in the distribution or quantity of 5-HT occurred after sacrifice. However, the tissues were kept cold with ice, and the dissection proceded in the same order for each experiment. The chloroform-ethyl chloride might have influenced the 5-HT levels in the dog experiments to some degree, although presumably the effect of the anesthesia would be constant for the control and experimental animals. It is unlikely that LSD-25 retained in the tissue extracts directly altered the response of the heart of Venus mercenaria in the 5-HT assay since the phenomenon of irreversible stimulation of

the heart by LSD-25, described by Welsh,[82] was not observed following treatment with any of the extracts.

It must be noted that the techniques utilized in these experiments do not allow one to generalize upon the results obtained. the findings at the times of sacrifice after LSD-25 administration, which were selected, do not necessarily bear any resemblance to results which might be obtained at other times. Similarly, the doses of LSD-25 which were utilized might not be optimal for demonstrating neurochemical changes. Significant redistribution of 5-HT might have occurred in brain areas which were not selected for analysis in this study. Moreover, redistribution of 5-HT following LSD-25 administration could occur on an intracellular level and escape detection.

Negative findings in a work of this kind do not exclude the possibility that LSD-25 acts centrally through a mechanism involving 5-HT. Such an action could take place without changes in 5-HT concentrations occurring in the brain which would be observable by the techniques employed.

On the other hand, the observation of alterations of 5-HT following LSD-25 administration, as suggested in the experiments using the dog's brain, imply a possible biochemical correlation with the activity and tolerance which follows LSD-25. Such a correlation cannot be established on the basis of present findings, but requires additional information concerning: 1. The interaction of LSD-25 with various chemical systems existing in the brain and 5-HT in particular; 2. neural receptor phenomena; 3. the neuro-

pharmacological actions of 5-HT; and 4. the relation of neural changes to behavior. Of these lines of investigation, the study of neurochemical systems appears to be the most promising at the moment. A great deal has been learned about the effect of certain compounds on the binding of 5-HT and on the enzymes concerned in its synthesis and destruction. The manner in which LSD-25 is involved in these processes, however, remains a problem for future investigation.

SUMMARY

Brain levels of 5-hydroxytryptamine (5-HT) were studied in rats at different time intervals following acute and chronic administration of lysergic acid diethylamide (LSD-25). No significant alterations of 5-HT content were noted at various times following a single LSD-25 injection. With chronic administration, a trend toward depression of brain 5-HT content after the second daily injection and slight elevation after the third, fourth, and fifth daily injections was observed.

In dogs, an unequal distribution of 5-HT within the brain was observed. No striking changes in 5-HT levels occurred following administration of LSD-25, with the possible exceptions of the midbrain, hypothalamus and amygdala, though the levels of all of the brain areas studied appeared to be slightly greater than the controls.

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